DEVELOPMENT OF A BRET BIOSENSOR-BASED SCREENING STRATEGY FOR IDENTIFYING DRIVERS OF HALLUCINOGENIC ACTIVITY OF 5HT2AR TARGETING COMPOUNDS

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INTRODUCTION

Promising clinical trial outcomes involving across various indications have stimulated a resurgence of scientific interest in compounds targeting the 5-HT2A receptor (5-HT2AR). However, many of these molecules act as hallucinogens, hampering their widespread therapeutic use. One goal in this field of research is the development of compounds that retain their therapeutic properties but lack hallucinogenic activity. Yet, there is no clear consensus on the key factors driving the hallucinogenic actions of 5-HT2AR agonists. In this work, we describe the application of novel enhanced bystander (eb)BRET-based biosensors towards the characterization and differentiation of hallucinogenic and non-hallucinogenic neuroplastogenic 5-HT2AR targeting molecules. These biosensors allow for the real-time and broad multiparametric monitoring of the signal transduction pathways engaged upon activation of native G Protein-Coupled Receptors (GPCRs). Using these tools, we can evaluate five core pharmacological factors that may help in distinguishing hallucinogenic from non-hallucinogenic neuroplastogenic molecules: pathway-specific compound efficacy / signaling bias at 5-HT2AR, compound polypharmacology, receptor activation kinetics, receptor internalization, and compound activity at subcellular compartments. We propose a biosensor-based and AI-supported screening pipeline in which various hallucinogenic and non-hallucinogenic 5-HT2AR agonists are first profiled on these five pharmacological parameters and the resulting data subsequently used to train machine-learning algorithms to cluster compounds based on their hallucinogenic activity. Novel 5-HT2AR targeting compounds can then be funneled through this pipeline to help isolate non-hallucinogenic compounds, and whose activity could then be validated in vivo. This approach, used in an iterative manner, may help inform the early identification and rational design of safer, non-hallucinogenic compounds for the use in various neuropsychiatric indications.

MATERIALS AND METHODS

ebBRET-based biosensor platform BioSensAll®

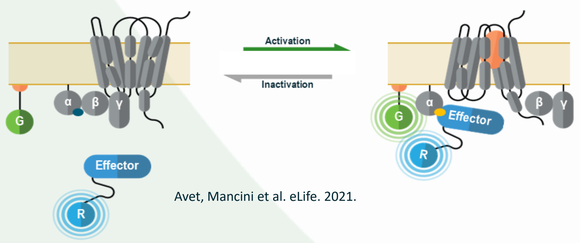


Figure 1: Assay principle underlying our ebBRET-based biosneors

Avet, Mancini et al. eLife. 2021.

RESULTS & CONCEPTUAL FRAMEWORK

Compound signaling bias & efficacy

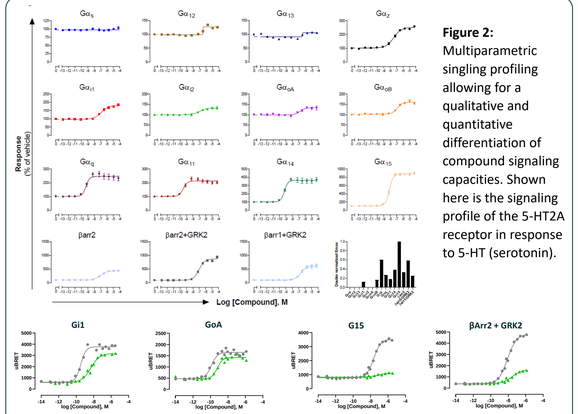
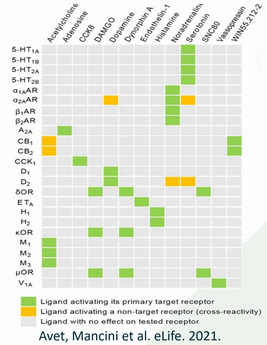


Figure 2: Multiparametric singling profiling allowing for a qualitative and quantitative differentiation of compound signaling capacities. Shown here is the signaling profile of the 5-HT2A receptor in response to 5-HT (serotonin).

Comparison of compound activity on various signaling pathways enabling the detection of inter-pathway differences in compound efficacy and functional selectivity. Differences in efficacy and functional selectivity may drive hallucinogenic activity of 5-HT2AR targeting agents (Wallach *et al.* DOI 10.1101/2023.07.29.551106. 2023; Karaki *et al.* Mol Cell Proteomics. 2014)).

Compound polypharmacology



Avet, Mancini et al. eLife. 2021.

Figure 3: Psychoactive substances often display activity at multiple GPCRs (i.e., polypharmacology). The ability to detect such promiscuity may allow to identify polypharmacological patterns that distinguish hallucinogenic from non hallucinogenic 5-HT2AR compounds. Shown here is the activity of various ligands on different receptors using our ebBRET biosensor assays.

Receptor activation kinetics

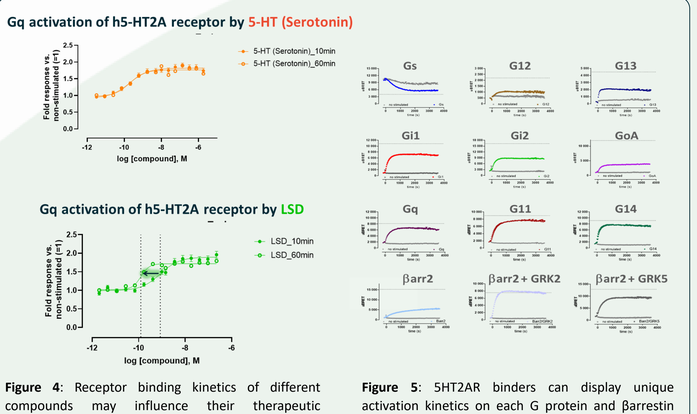


Figure 4: Receptor binding kinetics of different compounds may influence their therapeutic properties and their hallucinogenic potential. LSD's slow binding kinetics translates into a shift in potency. This may explain LSD's long duration of action and hallucinogenic activity *in vivo*.

Figure 5: 5HT2AR binders can display unique activation kinetics on each G protein and βarrestin pathway. Ligand-specific differences in pathway activation kinetics may translate into differences in hallucinogenic and therapeutic potential.

Compound activity at subcellular compartment

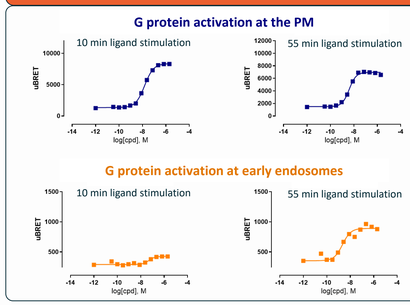
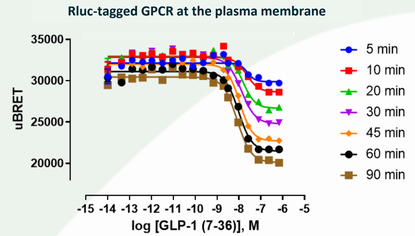


Figure 7: Detection of compound-induced receptor activity at intra-cellular compartments; It has been proposed that psychedelics promote neuroplasticity through the activation of intracellular (Golgi) 5-HT2AR (Vargas *et al.* Science. 2023).

Receptor internalization

Figure 6: 5HT2AR internalization is another parameter that can serve to differentiate various binders. Shown is our GPCR internalization assay measuring a time-dependent reduction of cells-surface GPCR levels.



Non-hallucinogenic

Hallucinogenic

CONCLUSIONS

- ebBRET-based biosensors can be applied towards advanced multiparametric pharmacological characterization of 5-HT2AR targeting agents.
- Measurement of 5 key pharmacological parameters, coupled with machine learning algorthims, can help quickly distingusih hallucinogenic and non-hallucinogenic 5-HT2AR binders.
- The proposed biosensor-based and AI-supported screennig pipeline can be used to identify and generate ligands with the desired psychoactive properties.